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TECHNICAL MANUSCRIPT 531

THE GRANULAR PNEUMONOCYTE:  
ABSENCE OF PHAGOCYTIC ACTIVITY

Charles S. Faulkner II  
John R. Esterly

MAY 1969

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Charles S. Faulkner II

John R. Esterly

Pathology Division  
MEDICAL SCIENCES LABORATORIES

Project 1T061101A91A

May 1969

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

#### ABSTRACT

Pulmonary phagocytosis has interested many investigators, but relatively few have used ultrastructural techniques for precise identification of pulmonary cells. The objective of this experiment was to determine the characteristics and variation in appearance of phagocytic cells under normal conditions and to observe the relationship between ingested particles and other cytoplasmic inclusions. A solution of India ink and polystyrene spheres was injected into the trachea of adult New Zealand rabbits. The animals were sacrificed after 45 to 60 minutes and specimens were obtained for light and electron microscopy. The particles were easily recognized in the airspaces, both free and in the cytoplasm of alveolar macrophages and occasional polymorphonuclear cells. Particles were often adjacent to granular or membranous pneumonocytes, but in no instance was ink or plastic identified within these epithelial cells, nor were particles found in interstitial or intravascular spaces. Lamellar inclusions were present in nearly all of the granular pneumonocytes (type II cell, great alveolar cell). In several samples, inclusions communicated openly with the alveolar space, but the observations do not support the hypothesis of a phagocytic origin of these cytoplasmic bodies. Phagocytosis in specimens from neonatal rabbits was not significantly different from that in the adult. These observations seem to indicate that the granular pneumonocyte is not a phagocytic cell, and that if it ever becomes phagocytic, it undergoes considerable change in its morphologic characteristics. No intermediate forms suggesting a transition from granular to phagocytic pneumonocyte have ever been observed by the authors.

### THE GRANULAR PNEUMONOCYTE: ABSENCE OF PHAGOCYTIC ACTIVITY\*

A definite alveolar epithelium was recognized and its cell types were described as a result of the magnification and resolution provided by the electron microscope.<sup>1-3</sup> There are two types of cells whose morphologic features are well known, although their origin and function are less completely understood. The membranous pneumonocyte (type I alveolar cell) is an attenuated squamous cell that lines the major portion of the alveolar surface. The granular pneumonocyte (type II alveolar cell, great alveolar cell) is more nearly cuboidal and often occupies corner positions in the alveolus. It has a vacuolated cytoplasm and electronmicrographs show characteristic lamellar inclusions that are thought by many investigators to be the precursor or storage form of pulmonary surfactant, the anti-atelectasis factor.<sup>4,5</sup> Available data suggest that both cell types are renewed by mitosis.<sup>6</sup> Nothing is known of the origin or transformation of one cell type from another, nor has a definite metabolic function been attributed to the membranous pneumonocyte.

In addition, the pulmonary septa contain capillary endothelial cells and reticuloendothelial cells. The latter appear as mononuclear leukocytes and histiocytes in septa and as alveolar macrophages in the airspaces. These cells are actively phagocytic and the majority are of bone marrow origin.<sup>7,8</sup> Their metabolism has been extensively studied, especially with respect to hydrolytic enzymes associated with phagocytosis and lysosomal activity.<sup>9,10</sup>

A recent report has suggested that lamellar inclusions in the granular pneumonocyte represent ingested rather than secretory material.<sup>11</sup> This study was undertaken to determine precisely which cells in the lung participate in phagocytosis, and to examine the cytologic features of these cells. It is a preliminary portion of a detailed study of phagocytosis and hydrolytic enzyme activity in the reticuloendothelial system.

Adult New Zealand rabbits were briefly anesthetized and 5 to 10 ml of a particulate solution were injected into the trachea. The solution was prepared by mixing, in proportions of 1:40, India ink and a 5% by volume suspension of 1-micron polystyrene spheres.\*\* The animals were sacrificed 45 to 60 min after injection. The portions of the lung into which the particulate solution had entered were easily distinguished by the India ink marker. Small fragments were fixed in phosphate-buffered 2.5% glutaraldehyde at 4 C. The specimens were subsequently osmicated, dehydrated, and embedded in Epon according to the technique of Luft.<sup>12</sup>

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\*\* Dow Chemicals, Inc.

Thick sections for light microscopy were stained with toluidine blue, and thin sections for electron microscopy were stained with uranyl acetate and lead citrate. Similarly processed tissues from normal-appearing areas of lung and from uninjected animals were included for comparison.

The various epithelial, interstitial, and free alveolar cell types were recognized and differentiated by location and ultrastructural features. Granular pneumonocytes were found resting directly on the alveolar basement membrane. As well as a moderate number of mitochondria, their cytoplasm contained numerous, similarly sized, rounded inclusions within which were varying amounts of a layered lipid material. The free border was formed by relatively uniform microvilli. The adjacent membranous pneumonocytes partially covered the sides of the granular pneumonocyte, and desmosomal areas of electron density were present near the alveolar side of the intercellular junctions. Alveolar macrophages, on the other hand, were not attached to the alveolar wall. Their irregular cytoplasmic projections varied considerably in size and shape and were clearly distinguished from the microvilli of the granular pneumonocyte. Multiple structures, including lysosomes, microbodies, and other larger, irregular structures containing various sorts of electron-dense material were present in the cytoplasm of the macrophage. Occasionally, the latter appeared as osmophilic, lamellated material resembling the inclusions of the granular pneumonocyte. We have not seen any instances, however, of a cell with the other characteristics of a macrophage, containing more than one or two such bodies and causing confusion with the granular pneumonocyte. Nor have we seen any of the irregular phagosomes, which are common in the macrophage, in any of the granular pneumonocytes. In practice, the distinction between the two cells was readily apparent (Fig. 1), and no transitional forms were recognized. In only rare cases, with tangential sectioning and insufficient cytoplasmic area, was identification difficult. Plasma cells, lymphocytes, and monocytes were identified in vascular channels, and neutrophils were present both in the blood vessels and, to a lesser extent, in the airspaces.

The injected particulate material was found free in the airways and within cells (Fig. 2). Multiple carbon particles and several plastic spheres were found in 90 to 95% of the macrophages, and in all of the less frequent intra-alveolar neutrophils. Carbon and plastic were also found in very close proximity to granular pneumonocytes, but particulate matter was never seen in these or in any of the other pulmonary cell types (Fig. 3). Nor was there other evidence of phagocytosis by granular pneumonocytes. In several fields, an inclusion of a granular pneumonocyte was observed in communication with an alveolar space, but even then, with the plastic and carbon in close proximity, particles were never found within the inclusion (Fig. 4).



FIGURE 1. Adjacent Granular Pneumocyte and Alveolar Macrophage, Illustrating the Cytologic Features that Allow Differentiation between the Two Cell Types in Most Instances (See Text). Enlarged from 9300X.

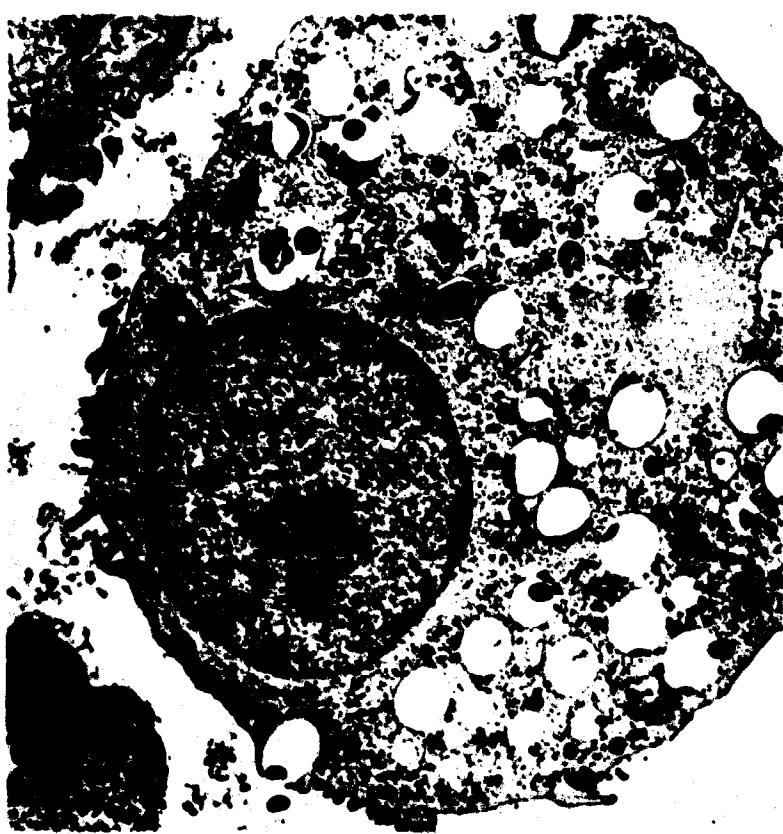


FIGURE 2. Alveolar Macrophage, Containing Abundant Plastic Spheres and Carbon Particles. One plastic sphere is being engulfed. 7000X



FIGURE 1. Granular Pneumocyte, Containing Relatively Few Inclusions, and a Macrophage Phagocytizing Plastic Spheres and Cells. Carbon appears to be in contact with the surface of the granular pneumocyte (arrow), but there is no evidence of phagocytosis by that cell. Estimated magnification,  $\times 12,000$ .

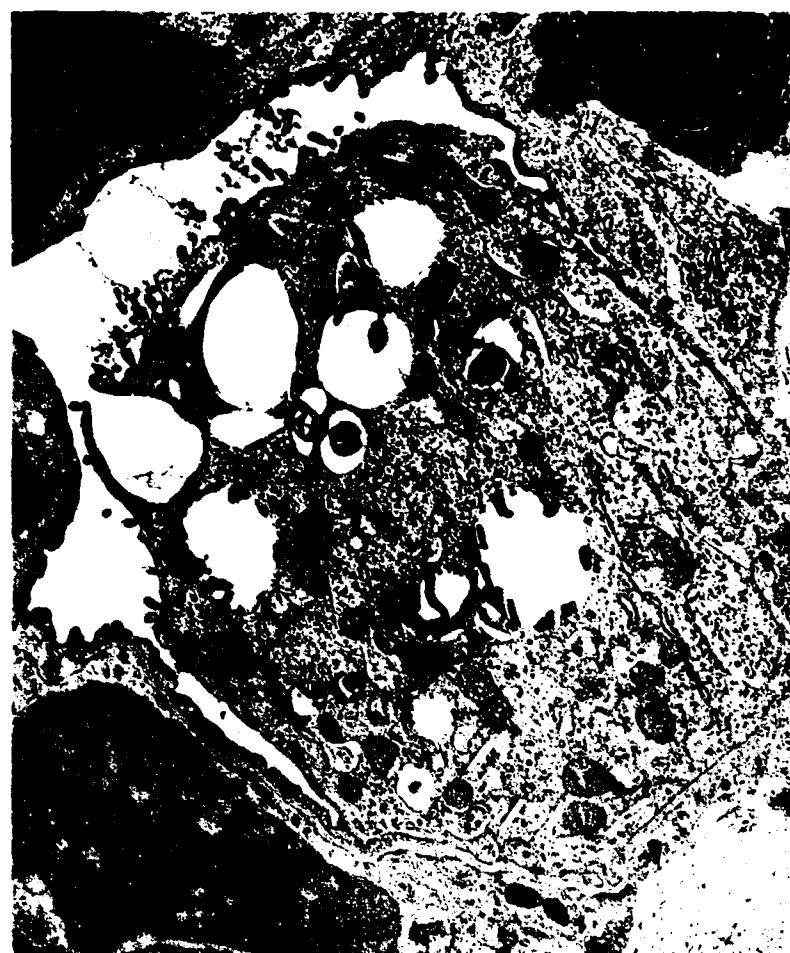


FIGURE 4. Granular Pneumonocyte, Illustrating Open Communication between the Interior of a Lamellar Inclusion and the Alveolar Space. Although carbon particles and a plastic sphere are near the opening, there is no evidence that the granular pneumonocyte is incorporating them. 13,200X

A third type of pulmonary epithelial cell (alveolar brush cell) described by Meyrick and Reid<sup>13</sup> was not recognized. Occasional granular pneumonocytes without lamellar inclusions were consistent with the description of brush cells, but they were never seen adjacent to other granular pneumonocytes, nor did they constitute even a portion of 10% of epithelial cells.<sup>13</sup>

Although it has been suggested that pulmonary epithelial cells have phagocytic capacity,<sup>14-16</sup> the only recent study with ultrastructural identification is the report of Niden. He described carbon particles in lamellar inclusions and concluded that the inclusions were a form of lysosome because of their acid phosphatase reactive membrane.<sup>11</sup> The findings in this study again demonstrate the active phagocytosis of alveolar macrophages but, within the experimental conditions, fail to confirm that the granular pneumonocyte is a phagocytic cell. Similar observations have also been reported in dogs exposed to aerosols of beryllium dust.<sup>17</sup>

Hydrolytic enzymes have been found in granular pneumonocytes by several investigations, but the observation is not necessarily evidence of lysosomal origin, since these reactions have been localized to secretory vacuoles and other cytoplasmic structures.<sup>18-21</sup> Furthermore, it has been argued that the lamellar inclusion is not phagocytosed material because it lacks a carbohydrate coat.<sup>22</sup>

The correlation of pulmonary stability and the presence of lamellar inclusions is based on a variety of circumstantial data, including appearance during gestation, experimental toxicity, and the localization of metabolic precursors to granular pneumonocytes.<sup>23-26</sup> Although precise characterization of pulmonary surfactant and its site of synthesis is lacking, these data implicate the importance of lamellar inclusions in the process, and are difficult to reconcile with their origin by phagocytosis. This study offers no additional evidence for the origin of surfactant, but it does indicate that particulate matter is not phagocytosed by the granular pneumonocytes, and it suggests that phagocytosis is an unlikely origin for lamellar inclusions.

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